The Theoretical Mechanistic Concept Of Sacogolottis Gabonensis, A Nigerian Alcoholic Beverage Additive As An Antioxidant Protector Against Hepatotoxicity

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Citation

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Abstract

The theoretical mechanistic concept of Sacoglottis gabonensis as a hepato-protector against xenobiotic oxidants has been correlated as follows:

a) Effect of Sacoglottis gabonensis on natural antioxidant defences during 2,4-DNPH-induced experimental membrane lipid peroxidation in vivo.

b) Effect of Sacoglottis gabonensis on metabolic and cytotoxic side effects of 2, 4-DNPH-induced peroxidation.

c) Antioxidnat effects of purpurogallin, -carotene and -tocopherol.

d) Antioxidant properties of bergenin - theoretically and experimentally derived.

e) Theoretical bioactivation of phenyl hydrazine.

f) Cytoprotective properties of Sacoglottis gabonensis and eugenol against hepatotoxicity.
The mechanism of the observed antioxidant property of the bark has been highlighted while validating its use in folkloric medicine practice.

INTRODUCTION

Natural plant products have been used in Nigeria especially in the tropical rainforest zone of Southern Nigeria in local medicine practice but very little of such substances have been subjected to scientific verification. This has limited their introduction and use in orthodox pharmaceutical preparation. The above not withstanding, available scientific data seem to favour a cytoprotective role for Sacoglottis gabonensis (Urban Humiriaceae) in hepatotoxicity and oxidant-induced experimental peroxidation reactions.

Sacoglottis gabonensis is a tropical rain forest tree that grows along the coastal region of the whole of West Africa. In Southern Nigeria particularly among the local dwellers in Abia, Akwa Ibom, Cross River, Delta, Bayelsa, Edo, Imo and Rivers State, the stem bark of this tree is used as an additive to palmwine, a local alcoholic brew which is an excudate from the phloem of raphia and palm trees (Okoye 2001). The stem bark prolongs the shelf life of the palmwine and reduces foaming and effervescence. It imparts a bitter taste to the sugary palm wine thereby, making the beverage more testable and acceptable with the amber colour. Claim are unanimous among consumers of the bark extract treated palmwine that it is used as a spice to serve as heating agent in nursing and pregnant mothers. One of the isolates of the extract was reported to be similar to chartreusis (Leach et al 1953). This result would tend to support, the earlier claim that the extract had been used in the treatment and management of microbial infections like gonorrhea. This would suggest a role in cytoportection and hence hepatotoxicity.

MATERIALS AND METHODS

EFFECT OF SACOGLOTTIS GABONENSIS ON NATURAL ANTIOXIDANT DEFENCES AFTER 2,4-DINITROPHENYL HYDRAZINE-INDUCED EXPERIMENTAL MEMBRANE PEROXIDATION.

The theoretical mechanistic concept of the hepatoprotective properties of Sacoglottis gabonensis stem bark extract will be demonstrated using the effects of the bark on natural antioxidant defenses after 2,4-DNPH-induced peroxidation in vivo. Using 2,4-DNPH as the primary xenobiotic oxidant, (Maduka, 2000, Maduka and Okoye, 2002) demonstrated that administration of the bark spared tissue depletion of the primary entioxidant enzymes, catalase and superoxide dismutase in liver and red blood cells. Though no significant effect was observed on the activities of glutathione peroxidase, another primary antioxidant enzyme which acts in aqueous phases to degrade H_{22} and organic peroxides, toxicological significant is not dependent on statistical significance (Afawodi and Maduagwu, 1992). Phenyl hydrazine is a long proven hemolytic poison as well as a hepatotoxicant. The above report shows that the bark extract of this botanical plant can play role in hepatoxicity as an antidote.

EFFECT OF SACOGLOTTIS GABONENSIS STEM BARK EXTRACT ON METABOLIC AND HAEMATOHEGICAL SIDE EFFECTS OF 2,4-DNPH-INDUCED TISSUE DAMAGE.

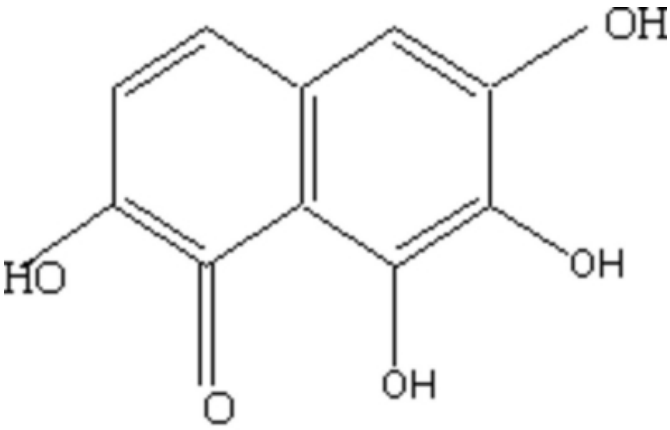
The administration the bark extract spared the tissue depletion of the haematological parameters, RBC, Hb, PCV and proliferation of WBC total and differential (Maduka et al, 2003). The status of those Physiological parameters are compromised during oxidative stress induced by oxidants such as 2,4-DNPH (Maduka et al 1999). The observed mechanism was by complementing red cell maturation factors. Metabolically, it was demonstrated in the same communication (Maduka et al, 2003) that ethanol induced energy over was arrested by treatment with the bark extract and its isolate, bergenin an isocoumarin of 2,1-benzopyrone. This appears to be a scientific evidence supporting the claims of local consumers of Sacoglottis gabonensis stem bark treated palmwine in the rainforest region Southern Nigeria. These reports also show that the bark extract will inhibit or reduce the rate of lipid peroxidtaion of membrane lipids.

ANTIOXIDANT PROPERTIES OF -CAROTENE, PURPUROGALLIN, -TOCOPHEROL AND EUGENOL.

Both endogeneous (usually enzymes) and exogeneous antioxidants are required to mop the free radical fluxes which accumulate due to metabolic or biological oxidations of the body (Machlin and Bendicvh 1987, Halliwell 1994).

Purpurogallin (2,3,4,6-tetrahydroxy-5H-benzocyclo hepten-5-one), a flavinol obtained from oak nutgalls prevented the lysis of human erythrocytes exposed to an initiator of peroxyl radicals, 2, 2-azo-bis(2-amidino-propane dihydrochloride) at 37°C.

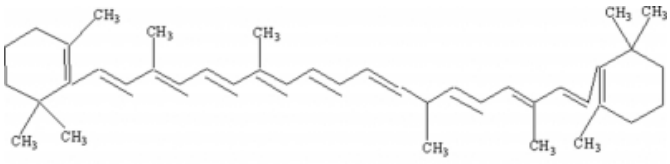
Figure 1



(Structure of Purpurogallin)

The inhibition observed with purpurogallin surpassed those of lactosylphenyl-trolox, trolox and ascorbate (Sugiyama et.al,1993).The mechanism involved appeared to involve the amphiphathic (both hydrophilic and lipophilic) nature of purpurogallin. B-carotene is an unusual lipid which acts in lipid phases to quench to quench free radicals in the lipid peroxidation pathway (Burton and Ingold, 1984).

Figure 2



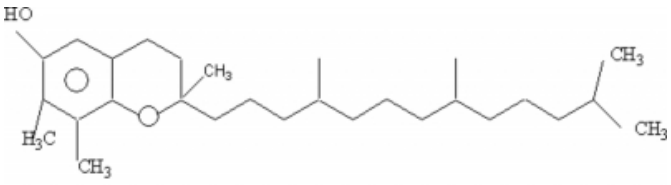
(Structure of B-carotene)

The compound contains an array of light absorbing substances like electrons. There is a systematic conjugation

of double bonds rich in electrons which aid the free radical trapping ability of B-carotene. Dietary B-carotene materially reduces human cancer rates (Peto et al, 1981).

The -tocopherol and its congeners contain light absorbing chromophores which are good in reducing the activities of free radicals. -tocopherol acts in lipid phases too to quench free radicals and other excited species generated during normal metabolic reactions. (Machlin and Bendich 1987). Evidence had been provided that free radical damage contributes to the etiology of many chronic health problems such as emphysema, cardiovascular diseases, cataracts and cancer.

Figure 3



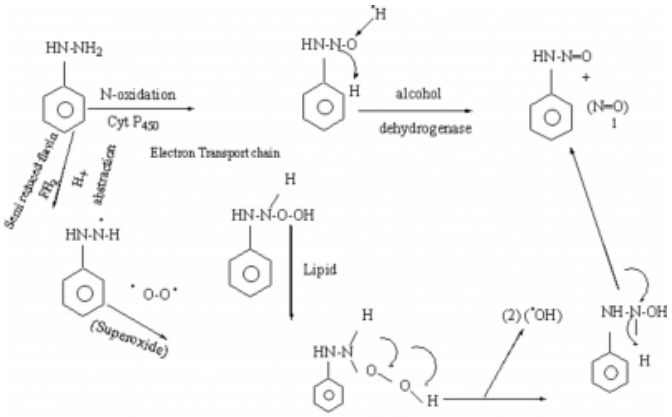
(Structure of α-tocopherol)

It was reported that the extent of tissue damage is the result of the balance between the tree radicals generate and the antioxidant protective defense system (Machlin and Bendich 1987). Since the liver is the main site for detoxidant protective defense system (Machlin and Bendich 1987). Since the liver is the main site for detoxication of foreign compounds (Lu and Kacew, 2002), any actioxidant principle which can aid or reduce the free radical fluexes in the body will play role in hepatoprotection. In thin regard, engenol, a 2-methoxy 4(2-propehyl phenol) an active principle of various plant extracts such as ocimum, clove and nutmeg exhibited hepatoprotetcive properties against CCl_4-induced toxicity in rats (Parasakthy et al 1993). Also Cyperus scariosus extract protected against acetaminophen and CCl_4-induced hepatotoxity (Gilani and Janbaz, 1995) in the same way as Cassia steberiana inhibited against hepatotoxity effects of acetaminophen in rats (Madusolumuo et al 1999). The endogenous antioxidant enzymes such as glutathione peroxidase, catalase, superoxide dismutase, glutathione transterase, glutathione reductase and glucose – 6 - phosphate dehydrogenase (Kumar et al, 1988) may not be enough to combat all the toxic insults of free radicals in the system. This emphasizes the need to source for exogenous antioxidants such as B-carotene, vitamins A, C and E, purpurogallin, eugenol and the like from dietary means to compliment the antioxidant roles of the endogeneous enzymic antioxidants.

THEORETICAL BIOACTIVATION OF PHENYLHYDRAZINE

The mechanism of phenylyhdrazine-induces peroxidataion will be employed by theoretical mechanistic biochemistry approach as reported (Akinfonwa 1991, 1992) to explain the theoretical bioactivation of phenylhydrazine using the scheme shown below: -

Figure 4



Theoretical mechanism of phenylhydrazine-induced peroxidation.

Clemens et al (1984) and Maduka (2000) reported that phenylhybrazine is a hepatotoxicant as well as a hemolytic poison. The toxic effects of phenylhydrazine had been attributed to its autoxidation, the subsequent oxidation of enzymes, membrane proteins and haemoglobin and its initiation of peroxidation reactions in membrane phospholipids. It was observed that some superoxide radicals may be involved in the toxic effect of phenylhydrazine (Clemens at al 1984).

Theoretical mechanistic biochemistry uses the structural nature of xenobiotic oxidants and their substituents to predict their reactivity in systems. This prediction is based on the nature of the resultant species of such compounds. From the reaction scheme shown above, phenylhydrazine can undergo either of two metabolic fates or pathways 1_a and 1_b. In the 1_a pathway, ph undergoes N-oxidation mediated by cytochrome P_{450} or electron transport chain to a nitroso compound, an active species which can be metabolized by alcohol dehydrognese to form compound 1 (N= 0). This compound can attack membrane lipids or DNA. The second pathway (1b) involves proton abstract (H^+), superoxide anions and the peroxyl radical which can attack membrane lipids to give the hydroperoxide hydroxyl anion ($^{\cdot}OH$). (2) is

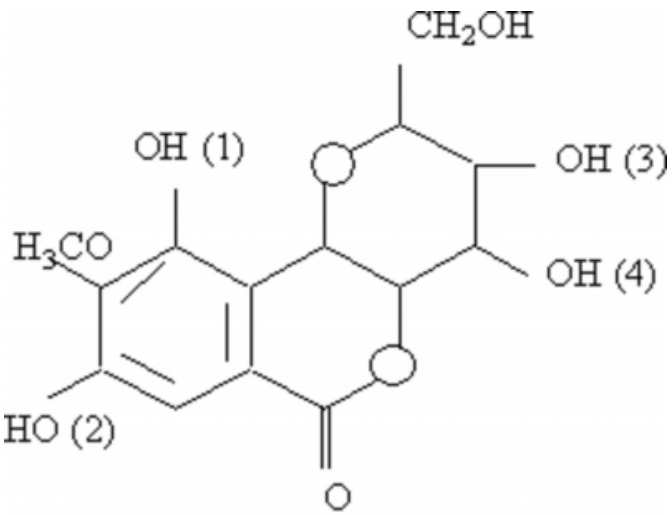
given off to give an alcohol which is metabolized by alcohol dehydrogenase to give compound (1), (N = O, nitroso intermediate). All the species generated via both pathways can peroxidize the membranes in the absence of any reductant which can quench the free radicals.

ANTIOXIDANT PROPERTIES OF BERGERNIN, AN ISOLATE OF SACOGLOTTIS GABONESIS STEM BARK EXTRACT.

The antioxidant properties of bergenin will be employed to demonstrate the efficacy of Sacoglottis gabonensis as a hepatoprotector against lipid peroxidants like phenylhydrazine. These will then be used to colloborate the theoretical mechanistic concept of Sacoglottis gabonensis as an antioxidant protector of cells against experimental membrane liquid peroxidation reactions.

Bergenin, a 2, 1-benzopyrone derivative of isocoumarin (C_{14} H_{16} O_9) was isolated from the stem bark of Sacoglottis gabonensis by Ogan (1971).

Figure 5



Structure of bergenin

From the above structure, positions 1, 2,3 and 4 are positions of antioxidant properties. From theoretical mechanistic biochemistry concepts, positions 1, 2>>3, 4 positions in antioxidant properties. Bergenin could give off any of the protons to react with the free radicals generated during the theoretical bioactivation scheme of phenylhydrazine shown. This would prevent or reduce the rate of lipid peroxidation in the lipid peroxidation pathway. It was also being observed in previous report (Maduka and Okoye 2000) that bergenin possesses hydrophilic and hydrophilic structures which would enhance its antioxidant protection of peroxidizing cells like red blood cells and liver.

In vivo, bergenin is being reported to protect against 2,4-dinitrophenyl induced hepatotoxicity and in red cells too (Maduka 2000, Maduka et al 1999). In vitro, bergenin also protected against peroxidative deterioration of stored vegetable oils over a period of time (Maduka and Okoye, 2002). The elegance of Sacoglottis gabonensis protection is further supported by later in vitro reports Maduka et al 2003) that the observed protection against peroxodation of stored vegetable oils compared favourably with vitamins C and E, two antioxidants of know mechanism of action employed in assessment of biological antioxidant properties of natural plant products.

In toxicological assessment, the rate of lipid peroxidation products corroborate the antioxidant status of the organisms. Recent reports have been made evaluating the effect of Sacoglottis gabonensis and bergenin on the formation of lipid peroxidation as well as complementing natural antioxidant defences (Maduka, 2004).

MECHANISM OF INHIBITION OF LIPID PEROXIDATION BY SACOGLOTTIS GABONENSIS AND BERGENIN IN THE LIVER

The stem bark of Sacoglottis gabonensis contains elements (Fe, Zn, Mn, Cu) which take part in oxidative reactions of antioxidant enzymes in the body (Maduka and Okoye 2002). Both the extract and bergenin, its isolate, Nigerian alcoholic beverage additives reduced the rate of formation of intermediates of the lipid peroxidation pathway (lipid hydroperoxide aldehydes, carbonyls) as well as complemented the primary antioxidant enzymes, catalase and superoxide dismutase during 2,4-DNPH – induced membrane lipid peroxidation is rat liver and red blood cells. The results of the studies are consistent, favouring conclusion that the extract and bergenin inhibited lipid peroxidation by the mechanism of inhibiting chain propagation and sparing tissue depletion of two primary antioxidant enzymes.

ANTI-HEPATOTOXIC PROPERTIES OF OTHER NATURAL PLANTS PRODUCTS

Some natural plant products have been used traditionally in the treatment of some liver diseases. Among them are Vitex domiana which protected the liver of albino rats from CCl4-induced liver damage by significantly reducing the activities

of serum alanine amino transferase,aspartate amino transferase, alkaline phosphatase, bilirubin and total protein (Ladeji and Okoye ,1996). Similarly, hot water extracts of legumes such as mung bean (Phaseolus radiatus), adzuki bean (Phaseolus aureus Roxb), black bean (Glycine max(l) Merr) and rice bean (Phaseolus calcaratus Roxb) showed antioxidant activities by demonstrating strong antilipid-peroxidation and superoxide anion scavenging activities (Lin et al, 2001).Argentine propolis extracts, used in folk medicine in Argentina protected against oxidative modification of lipid in unfractionated serum by inhibiting generation of malondialdehyde (Isle et al, 2001). The list is by no means exhaustive. In an earlier study, the antihepatotoxic effects of tannin analogues were examined utilizing CCl4 and galactosamine-induced cytoxicity in primary cultured rat hepatocytes. Most of the tannins like the hydrolysable tannins exhibited intense enzyme inhibitory action on glutamic pyruvate transaminanse while the condensed tannins exerted lower inhibitions in the two assay systems used (Hikino et al, 1985); this points to structure-activity relationships.

It means that the structure of any compound is important in its exhibition of cytoprotection and hence,antioxidant property. Certain compounds like enicatechin, gallate, B-Z-monogallate and HSF tannin showed strong antihepatotoxic properties but less enzyme inhibitory effects. This appears to mean that these compounds are promising antioxidans. It was also reported that certain lignins with a carbon skeleton closely related to diphenic acid (Hikino et al 1984) revealed intense antihepatotoxic properties. Combined knowledge indicated in the same report that substances with a partial structure of diphenic acid and also possessing some polyphenolic moieties contribute to their antihepatototoxic activities.

The action of Sacoglottis gabonensis is, therefore, clear. It contains a derivative of ethyl gallate as well as a bergenin, an isocoumarin derivative. Both have been associated with antioxidant properties in various published work both in and outside this laboratory. Bergenin has an amphipathic structural arrangement which will enhance its antioxidant properties in both lipid and aqueous environment.

The OH groups of positions 1 and 2 are stronger in antioxidant properties than those at positions 3 and 4. The structure is similar to those of B-Carotene,alpha -tocopherol, and purpurogallin, all free radical quenchers. The mechanism of action included inhibition of propagation and sparing antioxidant defenses pathways. The theoretical mechanistic concept approach favouring Sacoglottis gabonensis stem bark extract as an antioxidant with antihepatotoxic properties seems to have been well advanced after series of rigorous exercise. This report serves as additional support to justify its use in southern tropical rainforest Nigeria as a nutritional additive and in treatment of hepatotoxic disorders.

ACKNOWLEDGEMENT

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